## REMARKS

Claims 20 and 21 were previously pending and rejected. By this paper, new claims 22-24 are added.

Applicant is herein amending Claims 20 and 21 to correct minor clerical errors made when responding to the restriction requirement. Claim 20 as originally filed recites "SC-01MFP (Accession Number FERM BP-10077)" and Claim 21 as originally filed recites "SC-02MFP (Accession Number FERM BP-10078)", however in the subsequent responses, the order of Claims 20 and 21 were reversed. The current amendments simply correct the order of the two claims back to the original order.

New claim 22 is essentially the combination of the previously cancelled claims 4 and 7, plus some amendments for clarification and further limits the human cell strain to "a human myeloma cell strain".

New claim 23 is the new claim 22 plus the further limitation of the deposited cell strains SC-01MFP and SC-02MFP.

New claim 24 has the same preamble as Claims 22 and 23, which is limited by the deposited cell strains SC-01MFP and SC-02MFP.

No new matter has been added by the amendments.

**Claim Rejections under 35 U.S.C. §102**

The Office Action indicated that Claims 20 and 21 were rejected under 35 U.S.C. §102(e) as being anticipated by the articles by "Pene et al" and "Hata et al."

**Structural difference between the RPMI8226 and SC-01MFP cell strains, and between the KMS-12BM and SC-02MFP cell strains**

The examiner stated in the Advisory Action dated Dec. 24, 2008, "the burden is on applicant to show a novel or unobvious difference between the claimed product and the product of the prior art."

The RPMI8226 cell strain and the KMS-12BM cell stain, which are the starting cell strains of SC-01MFP and SC-02MFP respectively, are well known cell strains and many articles

have been written about their characteristics. However, their DNAs have not been mapped out at this point; therefore, there is no structural comparison study available for RPMI8226 vs. SC-01MFP, and KMS-12BM vs. SC-02MFP in term of DNA structure.

In the cited articles by "Pene et al" and "Hata et al", molecular makers which the RPMI8226 and KMS-12BM cell strains had expressed as the results of changing their incubation conditions were examined. The same experiments with the molecular markers could be done to SC-01MFP and SC-02MFP. However, it is just one of many possible experiments for many characteristics that these cell strains have, and even if such experiments demonstrate no difference between the original RPMI8226/KMS-12BM cell strains and the SC-01MFP/SC-02MFP cell strains, such results do not proof that there are no structural differences between them.

I nstead, Applicant is presenting herein the additional experimental data, which demonstrates the existence of the structural and functional differences between the RPMI8226 / KMS-12BM cell strains and the SC-01MFP / SC-02MFP cell strains.

The following two experiments were conducted:

Experiment 1: A recombinant gene vector was transfected into each of the SC-01MFP cell strain and its original cell strain RPMI8226, and the SC-02MFP cell strain and its original cell strain KMS-12BM, and subsequently each of the cell strain was cultured under the same incubation condition.

Results: While the SC-01MFP cell strain and the SC-02MFP cell strain maintained a stable production of the protein from the recombinant gene over 2 months period, their original cell strains RPMI8226 and KMS-12BM express the protein from the recombinant gene only for one-two weeks following the transfection.

Experiment 2: The SC-01MFP cell strain and its original cell strain RPMI8226, and the SC-02MFP cell strain and its original cell strain KMS-12BM were incubated in a serum free medium (ITES-RPMI1640 medium).

Results: RPMI8226 and KMS-12BM did not proliferate in the medium, while SC-01MFP and SC-02MFP proliferated.

**For the details of the experiments and results, please see the attached DECLARATION OF HIROHARU KAWAHARA.**

The results of the above experiments clearly demonstrate that the production method of the SC-01MFP and SC-02MFP cell strains described in the specification had modified the structures of the original cell strains RPMI8226 and KMS-12BM. If the assumption that no modification was caused by the production method was true and therefore the SC-01MFP and SC-02MFP cells were included in the original RPMI8226 and KMS-12BM cell strains, then, at least a small percentage of the RPMI8226 and KMS-12BM cells that underwent the experiments must have shown the same results as SC-01MFP and SC-02MFP, namely, the continuous production over 2 months period of the protein from the transfected gene, and some proliferation in the serum free medium. However, the experiments demonstrated that none of the original RPMI8226 and KMS-12BM cells showed the same results shown by the SC-01MFP and SC-02MFP cells. Therefore, these two experiments proof that there are structural and functional differences between the RPMI8226 and SC-01MFP cell strains, and between the KMS-12BM and SC-02MFP cell strains, and that the production method of the SC-01MFP and SC-02MFP cell strains does not simply screen out some of the RPMI8226 and SC-01MFP cells but actually transform them into cells with the two distinct characteristics that set them part from the original cell strains.

As stated above, the two distinct characteristics that SC-01MFP and SC-02MFP cell strains posse are:

1) the function of producing a protein from an exogenous gene which is transfected into the cells, at a yield of 1 ng – 10 μg/day per 1,000,000 cells at least over a 2-month period, and

2) the function to proliferate in a serum free medium.

Accordingly, Applicants request withdrawal of the rejection under 35 U.S.C. § 102(e) over "Pene et al" and "Hata et al."

**New Claims**

Applicant is herein presenting new claims 22-23.

The preamble of claims 22-23 is similar to the preamble of previously rejected and cancelled claim 7, but amended to limit "a human cell strain" to "human myeloma cell strain" and to clarify that the desired protein is from an exogenous gene.

In the previous office action, the examiner stated "the recited term in preamble merely reciting the intended use and does not result in a structural difference in the claimed human cell strain". However, as expressed in *Corning Glass Works v. Sumitomo Elec. U.S.A., Inc.*, 868 F.2d 1251, 1257, 9 USPQ2d 1962, 1966 (Fed. Cir. 1989), the determination of whether preamble recitations are structural limitations or mere statements of purpose or use "can be resolved only on review of the entirety of the [record] to gain an understanding of what the inventors actually invented and intended to encompass by the claim". The preamble of claims 22-24 defines what was actually invented: "A human myeloma cell strain, for the continuous production of a desired protein from an exogenous gene at a yield of 1 ng – 10 μg/day per 1,000,000 cells at least over a 2-month period". As stated in Page 2 of the present application, "At research levels, there are reports of producing protein by transfecting genes to human cells; however, the production period is at the level of approximately one month, thus being a transient result. So far, there is no example of success with a long and stable production period for industrial use." The inventor invented the cell strain of the present application in order to solve this problem. Therefore, the preamble should be interpreted as structural limitations rather than statements of purpose or use.

New claims 22 and 23 have the process step limitation which is disclosed in pages 6-8 of the present application.

The process steps are as follow:

a) selecting a human myeloma cell strain with a total intracellular protein in the range of about 0.1 – 1.0 mg per 1,000,000 cell; and

b) choosing cell clones of said human myeloma cell strain which have a doubling time of 18 to 24 hours and a 90% rate of cloning by limiting dilution method, adding a carcinogen to said cell clones, and selecting those cells which have a doubling time of 18 to 24 hours and a 90% rate of cloning by limiting dilution method" (Underlines sections indicate amendments to the original wording of the cancelled claims.)

The Office stated in the Advisory Action "the selection step of cell having cloning rate of over 90% do not contribute to making a mutant", and also "it is unclear what kinds of genetic changes were made by the presence of nitrosoguanidine since some population may undergo mutation while some population may not undergo mutation".

The original cell strains RPMI8226 and KMS-12BM do not have a cloning efficiency of over 90%. The cloning efficiency of RPMI8226 at the time it was delivered from a cell bank is 15%, and 24% for KMS-12BM. These rates do not vary much as long as the original cell strains were used (meaning not using the clones of the original cells). The selecting of the cells with a doubling time of 18 to 24 hours and a 90% rate of cloning by limiting dilution method may not cause mutation to the original cell strains. However, those highly proliferating cell clones that were screen out are already different from the original population, and definitely contributed to the acquisition of mutated cells which are capable of long-term protein production from an exogenous gene.

As for nitrosoguanidine, it has been used in cell culturing as a substance for inducing various mutations to cell lines in the past. Nitrosoguanidine does not affect a specific site of the gene of a cell, but it is known to affect a gene randomly, therefore regarded as a high probability mutation inducer.

The effectiveness and validity of the process steps are demonstrated by the experiment data presented herewith.

Lastly, Applicant wish to stress that **the order in which those process steps are carried out is the key for the creation of the cell stains of the present invention**. The following is a section of our remarks submitted to EPO with the demand for preliminary examination, which explains the importance of the particular order of the procedures/technologies used.

> "As the Examiner points out, the individual screening technologies are typical technologies, and are technologies that a person skilled in the art would naturally learn about. However, the cell strains of the present invention have been achieved for the first time through combining such individual technologies and from specifying such a threshold. Applicant believes that separately performing the individual technologies would not arrive at the accomplishments of the present invention.
> As stated above, a main aspect of the present invention is to provide cell strains having

the ability for long-term, stable production of proteins.

Thus, cell proliferation, and cell survival rates are the very first indispensable conditions. However, when screening with the aim of functions for protein productivity, if one selects, as the screening means, only cell strains having cell proliferation ability or high cloning efficiencies, a problem will arise.

This problem involves being able to acquire only clones having poor protein productivity (strains where production does not stabilize or strains that can only perform temporary production -- in other words, strains that do not have the aimed for properties).

Thus, the present inventors repeatedly performed trial and error experimentations to determine, for the individual screening steps, such aspects as their order, lengths of time and efficiencies, and how to combine them. Such aspects were determined with the aim of achieving the long-term, stabilized protein productivity, which is the purpose of the present invention. Accordingly, although each stage of such screenings is important, the present inventors further gained important knowledge regarding the order of such processes."

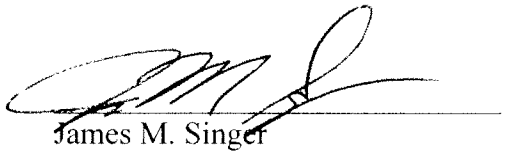
All of the stated grounds of rejection have been properly traversed, accommodated or rendered moot. Applicant therefore respectfully requests that the USPTO reconsider and withdraw all presently outstanding rejections. There being no other rejections, Applicant respectfully requests that the current application be allowed and passed to issue.

If the Examiner believes for any reason that personal communication will expedite prosecution of this application, I invite the Examiner to telephone me directly.

## AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for this Amendment and Response, or credit any overpayment, to deposit account no. 50-0436.

Respectfully submitted,
PEPPER HAMILTON LLP



James M. Singer
Reg. No. 45,111

Pepper Hamilton LLP
One Mellon Center, 50th Floor
500 Grant Street
Pittsburgh, PA 15219
Telephone: 412.454.5000
Facsimile: 412.281.0717
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